MATCHMAKER Library Construction & Screening Kit

A complete two-hybrid library construction and screening kit

- **Efficient—construct & screen a two-hybrid library in less than a week**
- **Practical—requires just 25 ng of poly A⁺ RNA**
- **Universal—generate two-hybrid libraries from any tissue source**
- **Simple & reliable—perfect for the first-time two-hybrid user**

In response to numerous requests, CLONTECH has developed the **MATCHMAKER Library Construction & Screening Kit**, a PCR-based system for building high-quality cDNA libraries in a GAL4-activation domain (AD) vector. This novel library construction protocol integrates the efficiency of our SMART™ technology with the sensitivity of our MATCHMAKER Two-Hybrid System 3. Using this powerful combination, library construction and screening can be completed in less than one week.

The MATCHMAKER Library Construction Kit relies on established techniques for the generation of complex two-hybrid cDNA libraries. Even researchers approaching this task for the first time will appreciate the dependable design of this system. Our protocol (Figure 1) features a novel, one-step *in vivo* cloning procedure that is both quick and efficient. This technique exploits the homologous recombination gap repair pathway in yeast to join DNA segments. Because the cloning step takes place *in vivo* in a MATCHMAKER yeast reporter strain, library construction and screening can be completed in rapid succession without the need for any bacterial transformation or amplification steps.

MATCHMAKER gets SMARTer

In designing the MATCHMAKER Library Construction Kit, we have paid particular attention to the method used for preparing cDNA. The quality of the cDNA used to build a two-hybrid library is vital to the success of your experiments. Should the cDNA synthesis procedure yield an unusually high number of incomplete cDNAs or if the procedure fails to copy certain transcripts, the result is a population of clones that lacks the complexity

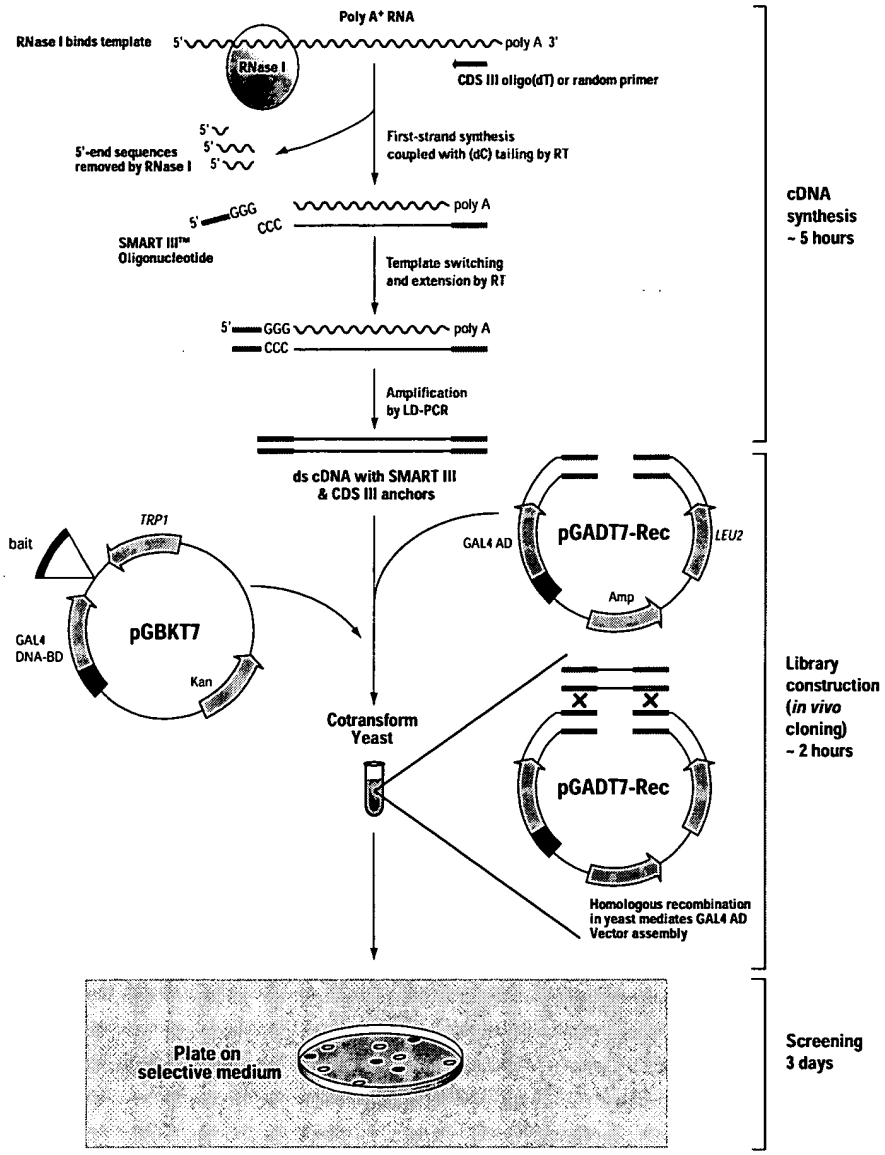

Figure 1. The MATCHMAKER Two-Hybrid Library Construction & Screening Kit procedure. Library construction begins with just nanograms of either total or poly A⁺ RNA. An oligo(dT) or random primer (CDS III) is used to prime the first-strand cDNA synthesis reaction, and RNase I is added to remove untranslated regions from the 5'-end of the transcript. When reverse transcriptase (RT) reaches the 5' end of the mRNA, the enzyme's terminal transferase activity adds a few deoxycytidines (dC). The 3' end of the SMART III Oligonucleotide anneals with the (dC) stretch, forming an extended template for RT. The resulting single-stranded cDNA is complementary to the mRNA template as well as the CDS III and SMART III oligos, which serve as universal priming sites for LD-PCR. Next, double-stranded cDNA, a linearized GAL4 AD vector (pGADT7-Rec), and a GAL4 DNA-BD/bait vector (pGBKT7) are cotransformed into a yeast reporter strain, where the cDNA recombines with homologous sequences in pGADT7-Rec. Transformants are then screened for two-hybrid interactions by plating on the appropriate selective medium. Your cDNA/AD fusion library can also be screened by mating with a pretransformed bait strain.

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MATCHMAKER Library Construction & Screening Kit...continued

of the original tissue. In screening such a population for interacting proteins, you are likely to miss important and potentially novel interactions. Fortunately, there is a method for generating high-quality cDNA while maintaining sequence representation: CLONTECH's SMART cDNA synthesis technology.

SMART, or Switching Mechanism at the 5' end of the RNA Transcript, is a cDNA synthesis and amplification technology that lets you produce a complete cDNA library from just nanograms of RNA. SMART is an essential component of a number of our cDNA synthesis products including the SMART™ cDNA Library Construction Kit. Now it has been adapted for use with our MATCHMAKER Two-Hybrid System 3 to construct two-hybrid libraries.

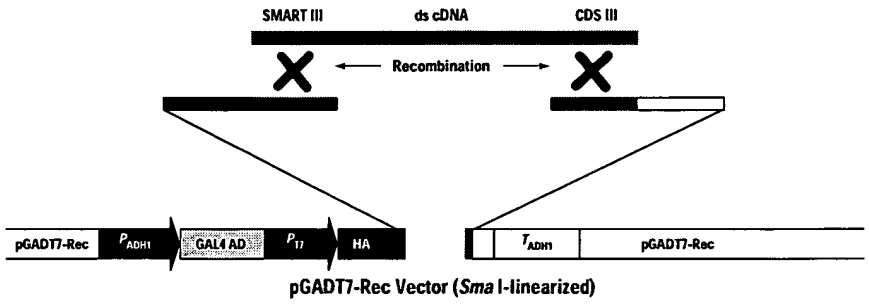

Figure 3. Large-scale transfer of cDNA to a GAL4 AD yeast expression vector, pGADT7-Rec, by homologous recombination. LD-PCR-amplified double-stranded cDNA is mixed with linearized pGADT7-Rec DNA (provided) and cotransformed into yeast cells. Homologous sequences at the ends of the cDNA are used by yeast repair enzymes to fill the gap in pGADT7-Rec, restoring the plasmid to its circular form. The outcome is a fully functional GAL4 AD/cDNA expression vector.

cDNA synthesis with SMART™

To meet the specific requirements of two-hybrid analysis, we modified the SMART procedure so that it is possible to remove noncoding 5'-end sequences from the mRNA template before they can be copied by reverse transcriptase. This step prevents 5'-end regions from being translated as GAL4 AD fusions.

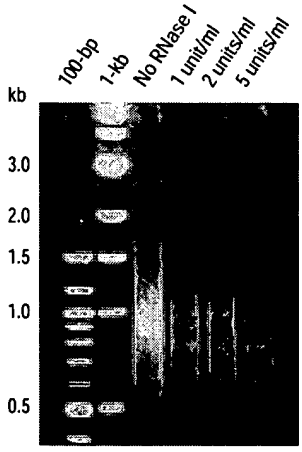

Figure 2. Noncoding 5'-end sequences can be removed from mRNA before two-hybrid library construction. RNase I degrades single-stranded RNA. When it is included in the first-strand cDNA synthesis reaction buffer, the enzyme removes 5'-end nucleotides before they can be copied to cDNA. As shown here, you can control the average cDNA length by changing the RNase I concentration.

These intervening sequences, not normally translated as part of the native protein, may block crucial binding domains, hiding them from interacting partners during the two-hybrid screen. With this modified procedure, you can remove 5'-end sequences by adding RNase I to the first-strand cDNA synthesis reaction (Figure 2).

We've built the MATCHMAKER Library Construction Kit around SMART because of its versatile design and economical protocol. SMART is versatile because it allows you to produce two-hybrid libraries from any tissue using total or poly A^+ RNA; it's economical because it lets you synthesize a library from just 25 ng of poly A^+ RNA. SMART has one other important advantage: it eliminates the need for adaptor ligation.

You don't need adaptors (or unique restriction sites) because cDNA synthesis and amplification are primed by the SMART III™ and CDS III Oligonucleotides. The CDS III oligo primes reverse transcription of poly A^+ RNA; the SMART III oligo attaches to the dC-rich cDNA tail—formed when our PowerScript™ Reverse Transcriptase (RT) reaches the end of the RNA molecule—and serves as an extended template for cDNA synthesis (Figure 1). After RT switches templates from the mRNA to the SMART oligo, a complete cDNA is synthesized with the additional SMART III sequence at the end. Double-stranded cDNA is produced and then amplified by long distance PCR (LD-PCR), the final step in creating a library of double-stranded cDNAs that contain SMART III and CDS III sequence anchors. With these anchors in place, cDNA can be transferred *directly* into the GAL4 AD yeast expression vector, pGADT7-Rec.

Let the yeast clone for you

The SMART double-stranded cDNA can be homologously recombined with pGADT7-Rec *in vivo* in a yeast reporter strain (Figure 1). Simply cotransform yeast with your SMART cDNA and the pGADT7-Rec vector (*Sma* I-linearized). Fast, convenient, and efficient, this one-step cloning procedure is possible because the SMART III and CDS III anchor sequences have also been engineered into the pGADT7-Rec plasmid. In its linear form, pGADT7-Rec is repaired in yeast by recombination with overlapping sequences at the ends of the SMART cDNA (Figure 3). Plasmid repair is manifest as a positive—i.e., as a $Leu2^+$ transformant. Cloning by this method is extremely efficient, yielding a complex collection of distinct clones (Figure 4). In two easy steps, you create a complete two-hybrid library, pretransformed and ready for screening, and all you need to provide is the RNA.

Library construction & screening in less than one week

With the MATCHMAKER Library Construction & Screening Kit, library construction and two-hybrid screening are seamlessly coordinated,

MATCHMAKER Library Construction & Screening Kit...*continued*

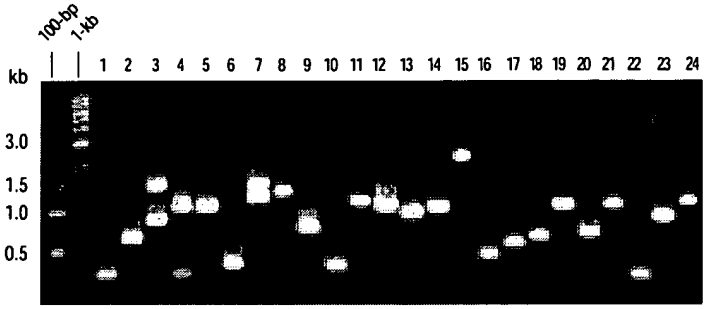

Figure 4. Rapid cloning by homologous recombination is extremely efficient and reliable. After cotransforming yeast with pGADT7-Rec (*Sma* I-linearized) and cDNA (prepared with the SMART method outlined in the text), transformants were selected on SD/–Leu plates. Randomly picked clones were screened for inserts by PCR. In this analysis, 24 out of 24 transformants carried plasmids with cDNA inserts. Note the wide range of insert sizes.

saving you time and effort. In fact, cloning and screening can be carried out in the same host strain on the same day! If you prepare a GAL4 DNA-binding domain (BD) fusion—e.g., pGBKT7/bait—in advance, you can include it in the cotransformation reaction together with your cDNA library and the pGADT7-Rec DNA. With a single transformation step, all three DNA components can be introduced into the yeast reporter strain (Figure 1). As soon as the pGADT7-Rec AD vector is assembled by the host's recombination processes, screening begins—automatically. Positive two-hybrid interactions can be identified immediately after transformation by plating transformants on medium that selects for the GAL4-responsive nutritional reporter gene.

MATCHMAKER vectors facilitate downstream characterization

The System 3 vectors pGADT7-Rec (a derivative of pGADT7) and pGBKT7, supplied with the MATCHMAKER Library Construction & Screening Kit, contain c-Myc and HA epitope tags, bacterial selection markers, and T7 promoters to help expedite the discovery and verification of new interactions. Inclusion of the epitope tags eliminates the need to generate antibodies to new proteins—CLONTECH'S c-Myc and HA antibodies (#3800-1 & #3808-1, respectively) will recognize fusion proteins *in vivo* and *in vitro*. The T7 promoter allows you to transcribe and translate epitope-tagged protein *in vitro* and also serves as a priming site for DNA sequencing.

Construct & screen your library one week, verify interactions the next

After identifying positive two-hybrid interactions using selective medium, researchers frequently evaluate their clones further using a variety of *in vitro* and *in vivo* biochemical assays. Our MATCHMAKER product line supports you in this logical next step. To test putative interactions outside of the yeast environment, try the pCMV-Myc and pCMV-HA Vector Set (#K6003-1), which allows you to confirm protein interactions by coimmunoprecipitation from mammalian cells.

It's easy to see why so many choose MATCHMAKER for their two-hybrid studies: library construction, screening, and analysis—MATCHMAKER covers it all.

For additional information on MATCHMAKER products, visit **matchmaker.clontech.com**

Product	Size	Cat#
MATCHMAKER Library Construction & Screening Kit	each	K1615-1

With every purchase of the MATCHMAKER Library Construction & Screening Kit, you receive a free trial-size Advantage™ 2 PCR Kit (#K1910-y), sufficient for 30 SMART PCR reactions.

Components

- **pGADT7-Rec Cloning Vector** (*Sma* I-linearized)
- **pGBKT7 (DNA-BD) Cloning Vector**
- **SMART III™ Oligonucleotide**
- **CDS III oligo(dT) and random primers**
- **PowerScript™ Reverse Transcriptase**
- **RNase I & RNase H**
- **Control Poly A⁺ RNA Template**
- **5' & 3' cDNA Amplification Primers**
- **First-Strand Buffer**
- **dNTP Mix**
- **CHROMA SPIN™+TE-400 Columns**
- **AH109 & Y187 Competent Yeast Cells**
- **PEG/ LiAc Solution**
- **Yeast Minimal Media Dropout Supplements**
- **pGBKT7-53 Control Vector**
- **pGBKT7-Lam Control Vector**
- **SV40 Large T PCR Fragment**
- **Complete User Manual** (PT3529-1)
- **Vector Information Packets** (PT3530-5 & PT3248-5)
- **Yeast Protocols Handbook** (PT3024-1)

Related Products

- **Advantage™ 2 PCR Kit** (#K1910-1, -y)
- **Advantage™ 2 Polymerase Mix** (#8430-1, -2)
- **SMART™ cDNA Library Construction Kit** (#K1051-1)
- **cDNA Libraries & Poly A⁺ RNA** (many)
- **HA-Tag Polyclonal Antibody** (#3808-1)
- **c-Myc Monoclonal Antibody** (#3800-1)
- **pCMV-Myc & pCMV-HA Vector Set** (#K6003-1)

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